חוק הפטנטים, תשכ"ז – 1967
PATENTS LAW, 5727-1967

בקשה לפטנט
Application For Patent

לשימוש הלשכה
For Office Use

090820	מספר: Number
30 JUN 1989	תאריך: Date
	הוקדם/נדחה Ante/Post-dated

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD., an Israel company, of 32, Hauniversita Street, Ramat Aviv, Tel Aviv 69975, ISRAEL

רמות רשות אוניברסיטאית למחקר שימושי ופיתוח תעשייתי בע"מ, חברה ישראלית, מרח' האוניברסיטה 32, רמת אביב, תל-אביב 69 975, ישראל

The inventors: Amihay FREEMAN
Ruth TOR

הממציאים: עמיחי פרימן
רות תור

בעל אמצאה מכח הדין – העברה – a right of law an assignment שמה הוא
of an invention the title of which is
Owner, by virtue of

(בעברית) (Hebrew): טרנסאסטריפיקציה בקטליזה אנזימטית עם אסטרים מונומריים של חומצה אקרילית ומתאקרילית

IL 90820

(באנגלית) (English): Enzymatically catalyzed transesterification with monomeric acrylic and methacrylic acid esters

hereby apply for a patent to be granted to me in respect thereof.

מבקש בזאת כי ינתן לי עליה פטנט

• בקשת חלוקה – Application of Division	• בקשה פטנט מוסף – Application for Patent Addition	• דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. מס'	No. מס'			
dated מיום	dated מיום			

• יפוי כח: בלבי עוד יוגש P.O.A.: general

filed on a previous case הוגש בענין קודם

המען למסירת מסמכים בישראל
Address for Service in Israel

DR. REINHOLD COHN AND PARTNERS
Patent Attorneys
P.O.B. 4060, Tel-Aviv C. 74921

חתימת המבקש
Signature of Applicant

For the Applicants,
DR. REINHOLD COHN AND PARTNERS
By: —

היום 29th בחודש June שנת 1989
This of the year

לשימוש הלשכה
For Office Use

15-03-1993
Publication date

טופס זה, המוטבע בחותם לשכת הפטנטים ומושלם במספר ובתאריך ההגשה, הנו אישור להגשת הבקשה שפרטיה רשומים לעיל.
This form, impressed with the Seal of the Patent Office and indicating the number and date of filing, certifies the filing of the application the particulars of which are set out above.

• בהתאם לבקשת הבעלים

טרנסאסטריפיקציה בקטליזה אנזימטית עם אסטרים מונומריים של חומצה אקרילית ומתאקרילית

Enzymatically catalyzed transesterification with monomeric acrylic and methacrylic acid esters

RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD.

רמות רשות אוניברסיטאית למחקר שימושי ופיתוח תעשייתי בע"מ

The inventors:

Amihay FREEMAN
Ruth TOR

הממציאים:

עמיחי פרימן
רות תור

C. 74921

FIELD OF THE INVENTION

The present invention concerns an enzymatically catalyzed process for the synthesis of acrylic and methacrylic acid ester monomors via transesterification reactions. Acrylic and methacrylic acid ester monomers including such that bear one or more hydroxyls, are currently employed in various polymcrization reaction, i.a. as co-monomers, in the preparation of hydrogels employed for the manufacture of contact lenses (Kirk-Othmer Encylopedia of Chemical Technology, 6, 728, 1979).

Recently several other applications were reported, including the synthesis of copolymers employed as plastic shields against neutron radiation (Fr. Patent 2455067), fixation of active compounds and drug carriers (J.C. Soutif, L. Ouchatar, D. Couvret, J.C. Bross, Makromol. Chem. 187, 561, 1986). In addition, such monomers may be useful for the preparation of improved gels for elctrophoresis, molecular sieving and immobilization of biocatalysts.

BACGROUND OF THE INVENTION

One of the major routes for the chemical synthesis of esters of acrylic and methacrylic acid monomers is transesterification according to the equation:

$$CH_2=C(CH_3)COOCH_3+ROH \longrightarrow CH_2=C(CH_3)COOR+CH_3OH.$$

According to this method a mixture of excess methyl methacrylate and the alcohol, catalyst and polymerization inhibitor are heated

at the reflux temperature and the methanol-methyl methacrylate azeotrope is removed overhead as it forms. Excess methyl methacrylate is removed by distillation and the product is isolated by distillation or other purification methods (Kirk-Othmer Encyclopedia of Chemical Technology, 15, 367, 1979). However, this approach does not allow for the synthesis of monomers based on diols, triols, or polyols as the substituent alcohol, as these polyfunctional alcohols will yield a mixture of mono with di, tri or poly acrylates and methacrylates.

Monomers bearing hydroxyl or dihydroxyl groups are therefore currently synthesized via other chemical routes. Thus, 2-hydroxyethyl methacrylate is made via the addition of methacrylic acid to ethylene oxide. However, the use of oxides other than ethylene oxide results in a mixture of products and commercial hydroxy propyl methacrylate, for example, is a 2:1 mixture of 2 hydroxypropyl methacrylate and 1-methyl-2-hydroxyethyl methacrylate (Kirk-Othmer Encyclopedia of Chemical Technology, 15, 367, 1979).

Enzymatic catalysis of transesterification reactions has gained much attention in recent years. Tranesterifications and interestifications of triglycerides, carried out in hexane by lipase immobilized on diatomaceous earth, were reported (for review see A.R. Macrae in "Biocatalysts in organic synthesis" J. Tramper, H.C. van der Plas and P. Linko, eds, Elsvier, p. 195, 1985). There have also been reported lipase catalysed

transesterifications of ethyl acetate, ethyl butyrate, trichloroethylbutyrate and tributyrine, all carried out with dried enzyme suspended in a water immiscible organic solvent (for review see A.M. Klibanov, Chemtech. 16, 354, 1986).

In two cases diols (e.g. 1,2 butanediol, 1,2 hexane diol) and sugar (e.g. glucose) were employed as the substituent alcohol (P.Cesti, A. Zaks and A.M. Klibanov, App. Biochem. Biotechnol. 11, 401, 1985; J. Chopineau, F.D. McCafferty, M. Therisod and A.M. Klibanov, Biotech. Bioeng. 31, 208, 1988), resulting in monoesters of acetic acid, propionic acid, butyric acid, caprylic acid and fatty acids derived from vegetable oils.

These above reported cases were significant departures from the previous prevalent opinion that enzyme catalysed reactions could only be performed in aqueous media. The purpose of that departure was to try and use enzymes for catalysing transesterification and interestifications reactions in which at least one of the reactants was synthetic.

The above described difficulties encountered in attempts to synthesize pure esters of acrylic and methacrylic acids bearing one or more free hydroxyl groups on the alcohol moiety of the ester, by conventional, non-enzymatic chemical reactions, make it essential to look for alternative method of production. Enzyme catalysed transesterification reactions in non-aqueous media of the kind described above, are unsuitable because of the lipophylic nature of the alcoholic reactants which requires that the envisaged transesterification reactions be

carried out in aqueous medium.

Accordingly, it is the object of the present invention to provide an improved process for the production of monomeric esters of acrylic and methacrylic acid via enzyme catalyzed transesterification.

SUMMARY OF THE INVENTION

In accordance with the present invention it has been found that enzymes that catalyse the hydrolysis of various esters in living organisms (animals, plants and microbial cells) are also capable of catalysing transesterification reactions, in aqueous based medium, between acrylic and methacrylic acid esters and various alcohols. This observation was unexpected as acrylic and methacrylic acid and their esters are synthetic substances and it was unpredictable that enzymes that normally operate in physiological surroundings of living organisms would be able to catalyse transesterification reactions in which the substrate and product are synthetic.

Based on this observation the invention provides a process for the production of esters of acrylic acid and methacrylic acids comprising reacting an ester of such an acid (starting ester) in aqueous medium with an alcohol ROH in which R is an organic radical other than the alcohol moiety of the starting ester, in the presence of an enzyme capable of catalyzing hydrolysis, esterification and transesterification reactions in living organisms (e.g. esterase, lipase).

Preferably the enzyme catalysed transesterification reaction according to the invention is performed with an excess of the alocohol ROH.

A typical enzyme that can be used for the purposes of the present invention is pig liver esterase.

The alcohol ROH used as a reactant in the transesterification reaction according to the invention, as well as the methanol – or ethanol – formed in the course of the reaction are liable to denaturate the enzyme that catalyses the reaction. Therefore, in accordance with a preferred embodiment of the invention, the enzyme is stabilised and optionally immobilised in accordance with the teachings of Israel Patent Application 83451. In accordance with these teachings, which are incorporated into the present disclosure by reference, enzymes are stabilised by encagement in a bi-layer protective structure comprising a polyaldehyde base coat linked to free amino groups of the enzyme, and cross linked therewith a polymeric outer coat made of a polymer which in the unlinked state comprises free amino and/or hydrazino groups. Optionally, such "encaged" enzymes may be further stabilised by immobilisation in a gel matrix with which it is cross-linked. These stabilised enzymes resist denaturation by alcohol and can accordingly be successfully employed for the purposes of the present invention.

The enzyme catalysed transesterification reaction according to the invention has the advantage of being specific. Accordingly, where the alcohol ROH comprises two or more free hydroxethyl groups, the esterification will occur only at one of these groups and accordingly a fully uniform product will be obtained, which is a great advantage over the prior-art chemical transesterification processes which in case of a di- or polyol reactant usually yield a mixture of different product esters.

The process according to the invention is carried out in an aqueous reaction mixture containing the substituting alcohol as the major component (50-95% v/v), buffer, the enzyme and the substrtate. The reaction generally takes several hours, depending on the concentrations and the temperatures employed.

DESCRIPTION OF THE DRAWING

The nature of the invention will be described hereinafter with reference to the annexed drawing which is a graphic representation showing the kinetics of enzymatic transesterification of ethyl methacrylate into hydroxyethyl methcrylate.

DESCRIPTION OF PREFERRED EMBODIMENTS

For better understanding, some specific embodiments of the invention will now be described in the following Examples, it being understood that the invention is not limited thereto.

Example 1

Enzymatic transesterification of ethyl methacrylate into 2-hydroxy ethyl methacrylate

A. Into a 20 ml glass vial 10 ml of a reaction mixture comprised of 70% (v/v) ethylene glycol (Merck, Cat. No. 9621) in 0.05M sodium phosphate, pH 7.3 were added, followed by the addition of 0.5 EU of pig liver esterase (E.C.3.1.1.1., Sigma Cat. No. E-3128) which was prestabilized and immobilized in glyoxal crosslinked polyacrylamide-hydrazide gel (according to R. Tor, Y. Dror and A. Freeman, Enzyme & Microbiol. Technol., 1990, 12 (4), 299-304. The enzyme and reaction mixture were incubated for equilibration for one hour at 37°C with shaking of 200 spm, and 25 µl ethyl methacrylate (Merck Cat. No. 800579) were added (final concentration: 20 mM), and the reaction allowed to proceed with shaking for 6 hours. Samples (1 ml) were withdrawn periodically, suspended material removed by centrifugation (2 min in Ependorf minicentrifuge 5414 S) and 0.3 ml of the supernatant was mixed with 0.7 ml methanol. The samples were analysed by reversed phase HPLC: 10 µl aliquots were assayed on a 12x0.4 cm Lichrochart 125-4 RP-8 column (Merck, Cat. No. 16052) employing 50:50 methanol-water containing 2.5% (v/v) acetic acid as the mobile phase, at a flow rate of 1 ml/min. Standards and samples were detected at 254 nm, employing commercially available 2-hydroxyethyl methacrylate (Merck Cat. No. 80058) as standard for the product. The kinetics of the enzymatic transesterification reaction under these conditions is shown in Fig.1. As shown in

Fig. 1 incubation of 6 hours resulted in the conversion of 82% of the substrate into 2-hydroxyethyl methacrylate. No methacrylic acid or ethyleneglycol dimethacrylate formation took place under these conditions.

B. The procedure described in part A above was repeated, as in Section A above, employing the same enzyme but immobilized without prestabilization. This enzyme brought about the conversion of 28% of the substrate within 6 hours of incubation into 2-hydroxyethyl methacrylate.

Example 2

Enzymatic transesterification of methyl methacrylate into 2-hydroxyethyl methacrylate

The procedure was similar as Example 1.A, employing 25 mM of methyl methacrylate (Fluka, Cat. No. 64200) as the substrate. Incubation for 6 hours under the conditions specified in Example 1 resulted in the conversion of 46% of the substrate into 2-hydroxyethyl methacrylate; ethyleneglycol dimethacrylate or methacrylic acid were not formed under these conditions.

Example 3

Enzymatic transesterification of ethyl acrylate into 2-hydroxyethyl acrylate

The procedure was similar as Example 1.A, employing 10 mM of ethyl acrylate (Fluka, Cat. No. 01750) as the substrate. Incubation for 6 hours under the conditions specified in Example 1, resulted in the conversion of 65% of the substrate into 2-hydroxyethyl acrylate (a commercially available standared

Aldrich, Cat. No. 29, 281-8, was employed to identify the product). Formation of ethylene glycol diacrylate or acrylic acid was not recorded under these conditions.

Example 4

Enzymatic transesterification of methyl acrylate into 2-hydroxyethyl acrylate

The procedure was similar as in Example 1.A, employing 7.5 mM of methyl acrylate (Fluka, Cat. 01800) as the substrate. Incubation for 6 hours under the conditions cited in Example 1.A resulted in the conversion of 40% of the substrate into 2-hydroxyethyl acrylate. Formation of ethyleneglycol diacrylate or acrylic acid was not recorded under these conditions.

Example 5

Enzymatic transesterification of ethyl methacrylate into 2-hydroxy propyl methacrylate

The procedure was similar as in Example 1.A, substituting 70% (v/v) propylene glycol (Fluka, Cat. No. 82282) for ethyleneglycol. Incubation for 6 hours under these conditions resulted in the conversion of 21% of the substrate into 2-hydroxypropyl methacrylate (commercially available standard, Aldrich Cat. No. 26,854-2, was employed to identify the product). In parallel, 9% of the substrate was hydrolyzed, generating methacrylic acid. Formation of propylene glycol dimethacrylate was not recorded under these conditions.

Example 6

Enzymatic transesterification of ethyl methacrylate into 2,3 dihydroxy propyl methacrylate (glyceryl mono methacrylate)

The procedure was similar as in Example 1.A, substituting 70% glycerol (Fluka Cat. No. 49770) for ethylene glycol. Incubation for 6 hours under these conditions resulted in the conversion of 72% of the substrate into 2,3 dihydroxypropyl methacrylate (commercially available standard Polysciences Cat. No. 4180 was emplyed to identify the product). In parallel, 28% of the substrate was hydrolyzed, generating methacrylic acid. Formation of glyceryl dimethacrylate was not recorded under these conditions.

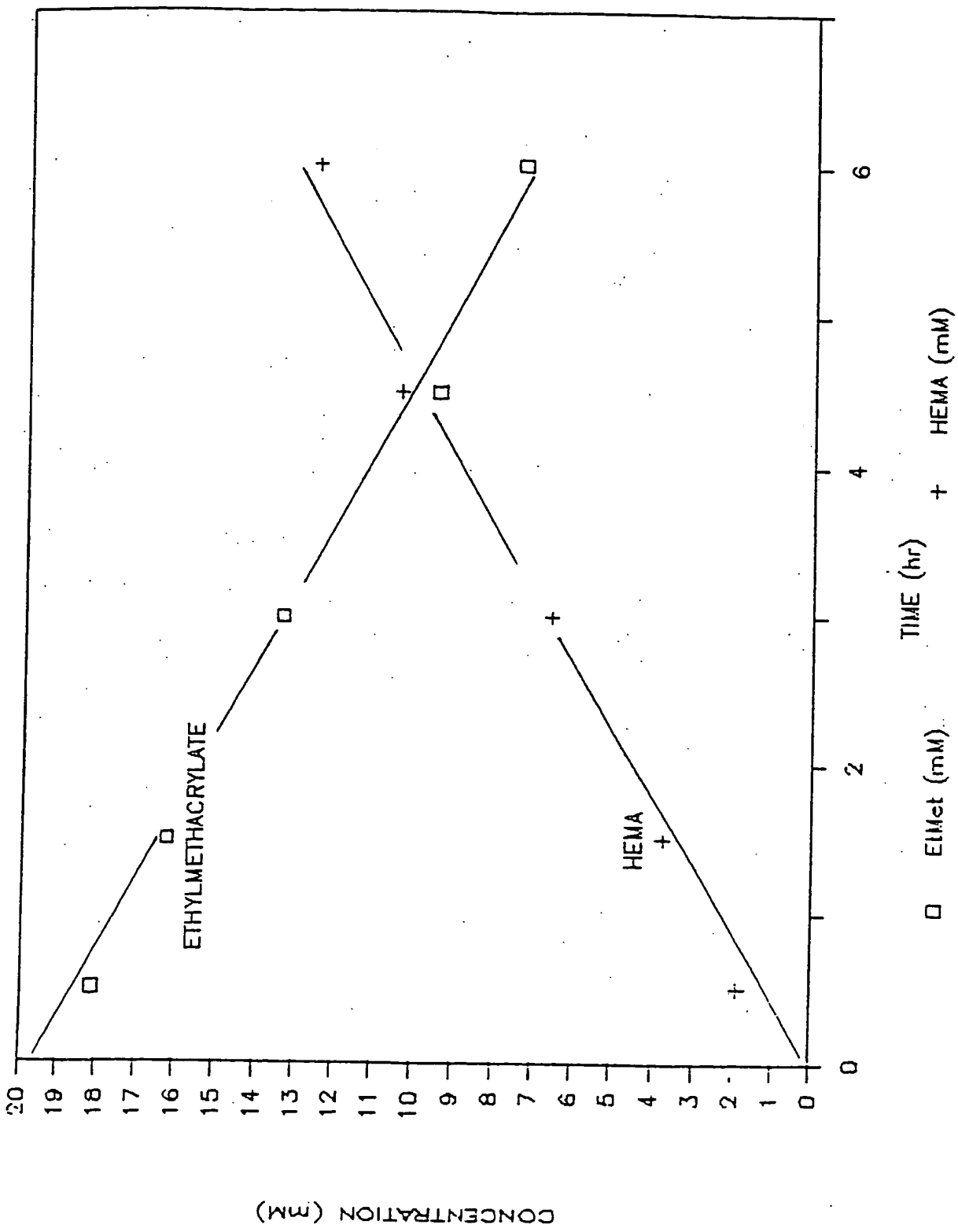
Claims

1. A process for the production of esters of acrylic acid and methacrylic acid comprising reacting an ester of such an acid (starting ester) in aqueous medium with an alcohol ROH in which R is an organic radical other than the alcohol moiety of the starting ester, in the presence of an enzyme capable of catalyzing ester hydrolysis, esterification and transesterification reactions in living organisms.

2. Process according to Claim 1, wherein the enzyme is pig liver esterase.

3. Process according to Claim 1 or 2, wherein said alcohol ROH is used in excess.

For the Applicants
DR. REINHOLD COHN AND PARTNERS
By:


ETHYLMETHACRYLATE
HEMA
CONCENTRATION (mM)
TIME (hr)
EtMet (mM)
HEMA (mM)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20